

GDCh-Advisory Committee  
on Existing Chemicals (BUA)

**Genistein**

**Model Substance for Describing  
Endocrine Effects of Phytoestrogens**

BUA Report 222

(February 2000)



S. Hirzel  
Wissenschaftliche Verlagsgesellschaft 2001

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edited by the GDCh Advisory  
Committee on Existing Chemicals

GDCh-Beratergremium für  
Altstoffe (BUA)



S. Hirzel  
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Dr. H. Behret  
Gesellschaft Deutscher Chemiker  
Postfach 90 04 40  
D-60444 Frankfurt am Main

Translated by P. Karbe

E-Mail: [bua@gdch.de](mailto:bua@gdch.de)  
Homepage: <http://www.gdch.de>

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## **Preface**

The Advisory Committee on Existing Chemicals of Environmental Relevance, BUA for short, was established in May 1982 to help the German federal government cope with the large task of dealing with existing chemicals. In an agreement between federal government, scientific community, and the chemical industry, it was associated with the German Chemical Society (GDCh-Gesellschaft Deutscher Chemiker) to ensure objective work, carried out in accordance with scientific principles.

At the end of 1997, the Committee was renamed 'GDCh Advisory Committee on Existing Chemicals' (abbreviation 'BUA' as before) and the statutes were revised to include EU level aspects of occupational safety for the handling of existing chemicals from then on. The collaboration with the Employment Accident Insurance Fund of the Chemical Industry (BG-Chemie), with its knowledge on workplace exposure and the toxicologic properties of chemicals, is a valuable addition to the BUA's know-how.

The cooperation between authorities, industry, and the scientific community, upon which the BUA is based, has proven worthwhile. No other national or international body has dealt with the ecological and health-related effects of so many existing chemicals as the BUA. On the national level, the BUA has produced comprehensive reports on about 300 substances and carried out preliminary evaluation and classification (priority-setting) for approximately 200 more, as of 1997. Publication of the process leading to priority-setting, in addition to the BUA Reports, lends transparency to the Committee's work.

Since the EU presently considers only those substances with a production volume of more than 1000 tonnes/year, the BUA began an additional national project in 1997, which also selects and assesses existing chemicals with a lower production volume in the range of 100 - 1000 tonnes/ year. The chemical industry presents about 50 databases for substances each year, for which the BUA sets the priority. Comprehensive reports are published on chemicals suspected of having a hazardous potential. If the data available for substance assessment are insufficient, the gaps in knowledge are documented and, if necessary, investigations recommended.

The BUA, as expert committee, increasingly addresses broad scientific questions and problems, such as 'endocrine disruptors', selection criteria for 'persistent organic pollutants' (POPs), 'risk assessment for soils and sediments', 'assessment criteria for the marine sector', and 'safety factors within the framework of toxicologic risk assessment'. The state of scientific knowledge on these subjects is researched, documented, and published as 'BUA Reports'. Through such scientific projects is a basis for an evaluation laid. The aim is to develop assessment approaches for the German federal government, determine gaps in knowledge, identify necessary research, and, last but not least, reduce information deficits in the general population. The present BUA report on the phytoestrogen Genistein is the result of intensive investigations and discussions on the theme of endocrine substances in the environment.

Munich, in March 2000

Helmut Greim  
BUA Chairman

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## Abbreviations

A	androgens (testosterone and 11-ketotestosterone)
ATD	1,4,9(11)-androstariene-3,17-dione
biochanin A	5,7-dihydroxy-4'-methoxyisoflavone
BW	body weight
CFTR	cystic fibrosis transmembrane conductance regulator
CI	confidence interval
coumestrol	3,9-dihydroxy-6H-benzofur[3,2-c][1]-6-one
daidzein	4',7-dihydroxyisoflavone
DDE	dichlorodiphenyl dichloroethene
DDT	dichlorodiphenyl trichloroethane
5 $\alpha$ -DHT	5 $\alpha$ -dihydrotestosterone
DES	diethylstilbestrol
DL	detection limit
DW	dry weight
E <sub>1</sub>	estrone
E <sub>2</sub>	17 $\beta$ -estradiol (1,3,5(10)-estratriene-3,17 $\beta$ -diol)
Equol	7,4'-dihydroxyisoflavane
ER	estrogen receptor
FGF	fibroblast growth factor
formononetin	7-hydroxy-4'-methoxyisoflavone
genistein	4',5,7-trihydroxyisoflavone
genistin	genistein-7 $\beta$ -glucoside
homogentisic acid	(2,5-dihydroxyphenyl)-acetic acid
i.p.	intraperitoneal
Jp	Japan
11-K	11-ketotestosterone
n	number of samples
n.d.g.	no data given
n.f.d.	no further data
n.g.	not given
NGF	nerve growth factor

NMDA	N-methyl-D-aspartate that binds to the glutamate receptors of the brain and is an agonist to the neurotransmitter glutamate
OH-PCBs	hydroxylated polychlorinated biphenyls
P	progesterone
phloroglucin	benzene-1,3,5-triol, dilospan S
PTK	protein tyrosine kinase
RI	relative inhibitory potential
RP	relative estrogenic potential
s.c.	subcutaneous
SD	standard deviation
SHBG	sex hormone binding globulin
T	testosterone
Tw	Taiwan
umbelliferone	7-hydroxy-2H-1-benzopyran-2-one, 7-hydroxycoumarin
VIP	vasointestinal peptide
VTG	vitellogenin
WL	water loss
WW	wet weight



# BUA Report on Genistein

## Summary

### Ecotoxicity

In agricultural ecosystems a decreased abundance (density of individuals in the population) has been proven for herbivorous mammals, caused mainly by the isoflavone formononetin. Due to the special properties of agricultural ecosystems, these observations cannot simply be transferred to ecosystems that are closer to nature.

The phytoestrogen content of pasture plants correlated negatively with various parameters relevant to reproduction (rates of reproduction, estrus, ovulation, and fertilization) and thus with the abundance of sheep. A reduced conception rate was attributed to formononetin or its metabolite equol. The enlarged teats of rams were shown to be caused by either formononetin or an isoflavonoid mixture, depending on the investigative conditions. An increased uterus weight was considered to be an effect of genistein.

Through the breeding of new types of pasture clover with a low formononetin content, the clinical symptoms of so-called "clover poisoning" have been avoided. However, infertility in sheep still leads to losses in agricultural productivity.

On the one hand, these observations are confirmed by metabolism investigations, which show that genistein, in contrast to formononetin, is degraded in ruminants by a special symbiosis with ruminal bacteria, allowing detoxification to occur. On the other hand, however, considering the more efficient detoxification capacity of sheep compared to cattle, the differences in sensitivity between these two animal species cannot be explained, and it must be clarified whether other factors, such as those related to receptors or morphogenetic effects of estrogens in the adult animal, could be the cause. The irreversible infertility observed only in sheep, as well as the

specific metabolism of ruminants, clearly indicate species differences and thus the fact that extrapolations between various animal groups and taxa are only of limited value.

In a field observation in a natural or nearly natural ecosystem, a connection was determined in birds between the reproduction rate and the content of isoflavones, including genistein, in their diet. This may represent a natural mechanism for regulating the abundance of the bird population through isoflavones. It remains to be clarified, however, whether and to what extent other mechanisms, such as nutritional deficiencies, also play a role. The difficulty also arises here of determining a reference population that is unaffected by hormonally active and possibly still unknown plant contents, and of distinguishing between exogenous influences on the endocrine system and the endogenous hormonal status. For birds, the effects observed in the environment were confirmed by *in vivo* experiments (start of egg laying, number of eggs), and the estrogen-mimicking effect was supported mechanistically by *ex vivo* investigations.

No results are available for reptiles.

For amphibians, only *in vitro* investigations are available, which do not allow an extrapolation to environmental conditions, precluding risk description for this class of vertebrates as well. Therefore it must further be clarified whether genistein, which interferes with the signal transduction mechanism caused by insulin and can affect osmotic regulation and oocytes, also leads *in vivo* to measurable effects in amphibians, and if so, whether the threshold concentration is present on an environmentally relevant scale.

For fish, it has yet to be determined whether the increased vitellogenin concentration observed in a breeding population and shown experimentally in *in vivo* investigations is of biological importance to the population. The relative roles played by phytoestrogens and the content of exogenous steroidal hormones in fish feed are not known either. It has been confirmed, at least for juvenile fish, that phytoestrogens

cause vitellogenin induction. The relative estrogenic potency of genistein was 2 to 3 orders of magnitude lower than that of estradiol, depending on the biological system investigated. In *in vivo* investigations, the factor was 2,500, measured on the basis of induction of the yolk protein vitellogenin, and in *in vitro* tests, 100, measured on the binding to the estrogen receptor, and 150 to 2,300, based on the induction of vitellogenin synthesis. Together with further evidence of the transactivation of genes controlled by the estrogen receptor, this confirms that, in fish, genistein can have an estrogen receptor-mediated, estradiol-agonistic effect. Since aromatase inhibition occurs only at high genistein concentrations and the competitive binding of genistein to the sex hormone-binding globulin was weak or not measurable, compared to that of the endogenous sex hormones, the effect on the biosynthesis of estrogens and any change occurring in the active concentration of sex hormones, caused by the binding to the sex hormone-binding globulin, are probably of minor importance. The effect on the osmotic regulatory glands measured in *in vitro* tests is probably not relevant, due to the comparatively weak active potential of genistein *in vivo*.

The effects of genistein described for invertebrates are presumably due to the inhibition of tyrosine kinase activity. It should be mentioned here that an effect was shown *in vitro* on neuro-endocrine cells which control reproduction in mollusks.

Genistein is an example of a substance with several modes of action. It interferes, for example, with both the signal transduction mediated via steroid receptors and with signal transduction mediated by some membrane receptors and based on the inhibition of tyrosine kinase activity. On the one hand, the estrogen-like effect of genistein was shown for farm animals in the field, as well as experimentally *in vivo* and *in vitro*, as described in the toxicology section. This mechanism has also been confirmed for birds *in vitro* and for fish in *in vivo* and *in vitro* experiments. On the other hand, based on *in vitro* test results for various animal groups, e.g. amphibians and mollusks, interaction has been proven regarding signal transduction induced by the nerve growth factor and that induced by insulin.

Additional modes of action are the inhibition of topoisomerase II (Corbett et al. 1993, for diptera) and the effect on membrane transport systems (amphibians, fish).

An assessment of the substance and its endocrine effects is hampered by the lack of data on the accumulation and metabolism in poikilothermic animals. Also missing are field studies with feral herbivorous vertebrates and experimental *in vivo* investigations on the simultaneous effects of genistein and other phytoestrogens.

Data on the concentration-effect relationship are available from only one *in vitro* test. NOEC/LOEC values for an endocrine effect of genistein have not been reported. It should be taken into account that the question of a threshold value for endocrine active substances is still open (National Research Council 1999; Sheehan et al. 1999).

Available data indicate genistein to be biodegradable. Since genistein can also be metabolized, at least in mammals, it presumably does not accumulate and a relevant exposure is probably limited to terrestrial wild animals feeding on certain plants. Particularly areas with single-crop farming and animals that are restricted in their choice of habitat and forage could be affected.

Sufficient information is not available on the occurrence of a relevant exposure of aquatic organisms to genistein. In soy processing most of the aglycones (including genistein) enter the rinsing water and wastes (Wang et al. 1998 b). Thus, local introductions into surface waters cannot be excluded. Genistein excreted in the urine and feces (of farm animals) may also enter the wastewater and spreading manure. No test results are available on genistein contents in waters or on population experiments with aquatic vertebrates (fish, amphibians) and invertebrates (mollusks, crustaceans) exposed to genistein. Investigations on sturgeon (fish farming) have shown that high phytoestrogen or steroid contents in feed can lead to increased vitellogenin-plasma levels. On the other hand, considering the weak estrogenic potential of phytoestrogens in relation to synthetic or endogenous steroids, any introduction of genistein into aquatic ecosystems would probably have comparatively

minor effects on aquatic organisms, if at all (cf. Purdom et al. 1994, Sumpter et al. 1996). No systematic investigations are available on this subject, however.

Genistein is considered to be biodegradable; data on bioaccumulation are not available. Based on the available data, one cannot determine whether joint exposure to genistein and other phytoestrogens, through the use of commercial feed containing soy in industrial farm animal production, fish farming, or laboratory animal husbandry, might lead to production losses.

The natural occurrence of genistein in terrestrial vascular plants, and the presence of various compounds in plants that are structurally similar to sex hormones and show estrogen-like effects, support the view that the described population-relevant effects may represent a long-term defense mechanism which protects plants from being eaten by animals.

Thus, it may be said that estrogenic effects of biological relevance to the population have been caused by phytoestrogens in agricultural ecosystems and in an ecosystem closer to nature. The plant-animal interaction described in nature may represent a natural regulatory system of population dynamics.

## Toxicity

Sharpe and Skakkebaek (1993) presented the hypothesis that human exposure to substances with an estrogen-like effect has steadily increased in recent decades and associated this with reports on increased incidences of organic and functional disorders in male reproduction organs. Accordingly, exogenous substances with estrogen-like effects are said to cause irreversible damage during sensitive stages of fetal development, which would later be manifested in the form of various disturbances. The authors suspect the involvement of environmental chemicals with estrogen-like effects and phytoestrogens. Various authors and committees (BUA 1999, CSTE 1999, Degen et al. 1999, Eisenbrand et al. 1998, Greim 1998 a, Safe 1995) conclude, on the basis of available data, that environmental chemicals with estrogen-like effects probably have a minor influence on human health and may be less important than phytoestrogens.

The present report is concerned with the importance of phytoestrogens, using genistein as the model. The substance occurs at high concentrations in soybeans and is therefore of great practical significance for human exposure in Asia, in particular. In western industrial countries as well, however, soy products are of increasing importance as food substitutes and additives. Epidemiological data associate soy products with an anti-cancer effect, also attributed to genistein.

In man, pharmacokinetic and metabolic processes of genistein are partly determined in essential stages by enzymatic reactions of the intestinal flora, and have yet to be clarified completely. As far as one can judge today, genistein is rapidly absorbed, metabolized, and eliminated along renal and biliary pathways. In the organism, degradation occurs mainly in the liver via glucuronidation, and thus, due to the "first pass effect", the systemic concentration is assumed to be low. Re-absorption in the enterohepatic system is probable. The substance is not persistent, however, and, depending on the dose, is eliminated within a few hours or days. With a traditional diet, the plasma levels of Asians are about 300 nM genistein, far higher than the approximately 6 nM of Western Europeans (Adlercreutz et al. 1993). The highest

plasma levels of up to 4,000 nM were found in infants who received soy-based milk-substitution formulas (Setchell et al. 1997).

Both *in vitro* investigations and animal experiments have shown genistein to have estrogen-like effects. Differences in the pharmacokinetics after oral or parenteral uptake suggest that the effect differs in strength for the various uptake pathways. Despite the markedly lower affinity to the estrogen receptor  $\alpha$  compared to estradiol, and the weaker effect in *in vitro* investigations and animal experiments, estrogen-like effects can occur in the human organism, due to the markedly higher plasma level of genistein in relation to estradiol. Genistein probably interacts with the recently discovered estrogen receptor  $\beta$  as well, which is distinguished by a different organ distribution and a considerably higher affinity for some phytoestrogens, especially genistein.

For example, minor effects on the hormone system and an extension of the menstrual cycle of premenopausal women were found at isoflavone or genistein concentrations corresponding to the usual Asian diet. Such effects could very well have a positive influence on the cancer incidence, especially with regard to breast cancer; however, they cannot be definitely determined epidemiologically or attributed to genistein or a soy-based diet. These effects are not considered to be adverse in character; in contrast, numerous studies are available on the health-promoting effects of genistein and phytoestrogen-rich diets in man. On the other hand, the taking of highly dosed preparations is not advised (Przyrembel 1998), as systematic investigations on possible undesirable effects are not available.

## Research Requirements

The risk to aquatic organisms of increased genistein exposure cannot be assessed on the basis of present knowledge. This would require further investigations on exposure and population experiments, for example with fish and crustaceans.

Although no adverse or estrogen-like effects of soy-based, milk substitute, infant formulas have been reported to date, systematic investigations on the consequences of such high exposure are not available.

No data are available on the *in utero* exposure of the fetus to phytoestrogens from the maternal diet. So far, no indications of disturbed sexual development that could be attributed to prenatal exposure to phytoestrogens are known for the offspring of Asian mothers with traditional eating habits.

Thus, while available case reports on human exposure to genistein and other phytoestrogens provide no confirmation of the hypothesis formulated by Sharpe and Skakkebaek (1993), systematic investigations on this subject are lacking.

In contrast, animal experiments have indicated that, especially with early postnatal and *in utero* exposure, genistein may affect certain processes important for sexual development. An assessment of the observed effects is limited, however, by the unphysiological uptake pathway (s.c.) selected in most of the studies. Since only isolated endpoints were investigated in each case, the biological relevance of these results is unclear. An, at least, partial clarification of open questions is expected from a 3-generation study with genistein in Sprague-Dawley rats, presently in progress (Delclos 1999). In this study, all the endpoints generally required for toxicological studies are being investigated, which should allow the determination of any functional or structural disorders of the reproductive organ due to genistein.